Table 2. Effect of rheumatoid factor (RF) on the neutralization of virus sensitized with human IgG.

Reaction mixture		Titer of virus in surviving fraction (PFU/ml, log 10)	Percentage of virus in surviving fraction neutralized by		
Human anti-HSV IgG (dilution)	Human IgM*		Anti-human IgG	Anti-human IgM	Human complement
1:25	Non-RF†	4.4	95	20	37
1:25	RF	4.3		94	95
1:50	Non-RF†	5.0	97	20	20
1:50	RF	5.0		96	92
1:100	Non-RF†	5.7	60	20	20
1:100	RF	5.4		60	50
1:200	Non-RF†	5.8	37	20	0
1:200	RF	5.8		20	0
Control‡	Non-RF†	5.8	0	20	0
Control‡	RF	5.8	0	20	0

* Diluted 1 : 5 in all reaction mixtures. † IgM from nonrheumatoid patient. ‡ IgG (diluted 1 : 25) from patient lacking antibody to HSV.

from an individual lacking both RF and antibody to HSV served as the source of complement. The data in Table 2 show that infectious complexes composed of HSV and human anti-HSV IgG were not susceptible to neutralization by anti–human IgM and only slightly susceptible to neutralization by human complement. After incubation with RF, however, the infectious complexes were highly susceptible to neutralization by both anti–human IgM and human complement. The amount of neutralization produced by anti–human IgM and complement was related to the concentration of anti-HSV IgG used to sensitize the virus.

Our experiments showed that incubation of RF with infectious HSV-IgG complexes failed to produce neutralization. However, the demonstration that specific anti–human IgM was able to neutralize infectious complexes that had been incubated with RF showed that RF had attached to these complexes. Moreover, the newly formed infectious HSV-IgG-RF complexes were quite susceptible to neutralization by complement. Our findings suggest that the interaction of RF with IgG provided new sites for the attachment of complement (*10*) and that increased coverage of the surface of the virion by the components of complement was probably responsible for neutralization (*4*). The ability of RF to enhance virus neutralization in the presence of complement suggests that RF might play a role in the host's reaction to virus infections.

Although in our experiments RF enhanced complement-mediated neutralization, it is possible that RF might be inhibitory to the complement-mediated neutralization of viruses sensitized with different classes or quantities of immunoglobulins. Schmid *et al.* (*10*) showed that in the red blood cell system whether RF enhanced or inhibited complement fixation depended on the concentration of sensitizing IgG. Recently several investigators reported that RF interfered with the fixation of complement by certain viruses (*11*). Whether complement competitively interferes with the fixation of RF remains to be determined. Apart from the role of RF in virus neutralization, the demonstration that RF or complement or both can attach to VA complexes suggests that not only VA complexes (*2*) but VA-complement complexes, VA-RF complexes, and VA-RF-complement complexes should be considered in the etiology and pathogenesis of immune-complex disease.

WARREN K. ASHE, CHARLES A. DANIELS
GEORGE S. SCOTT
ABNER LOUIS NOTKINS
Virology Section, Laboratory of Microbiology, National Institute of Dental Research, Bethesda, Maryland

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20 November 1970

Transneuronal Transfer of Radioactivity in the Central Nervous System

Abstract. *After injection of tritiated amino acid into the mouse eye, radioactivity appeared in the contralateral visual cortex, indicating that some material had been transferred from optic axons to lateral geniculate neurons. The radioactivity in the cortex was about 2 percent of that arriving in the geniculate, and most of it was contained in material that appeared to be protein.*

The idea that materials may pass from one nerve cell to another is closely related to the concept that the neuron has a "trophic" action on other cells, an action which enables it to "initiate or control molecular modification in the other cell[s]" (*1*). In demonstration of this trophic effect, it has been shown that, when a nerve fiber degenerates, its postsynaptic elements (or, in the case of a sensory nerve fiber, its sensory receptors) become severely deranged in both structure and function, and sometimes even disappear (*2*). For example, removal of the eye, which causes degeneration of the primary optic fibers, is followed by atrophy of the cells of the lateral geniculate body on which the optic fibers end (*3*). There is some indirect evidence that the trophic effects are mediated by the release from the nerve cell of substances other than the usual synaptic transmitters (*1*).

Direct evidence for the transneuronal

transfer of such substances has been meager. Even after presynaptic nerve endings have been heavily loaded with radioactive protein, there is no large-scale transfer of radioactivity into the adjacent postsynaptic cell bodies (*4, 5*). Nevertheless, a few provocative reports have appeared showing that some radioactivity may pass from nerves to muscle cells, to sensory receptor cells, or to contiguous neurons (*6*). Unfortunately, the radioautographic techniques used in these studies are difficult to evaluate quantitatively, and until now no preparation has been developed which is suitable for the measurement and identification of the transferred materials.

I have looked for evidence of transneuronal transfer in the optic system of the mouse by examining the visual cortex after injection of labeled amino acid into the eye. As in every species thus far examined, the injected amino acid enters the retinal ganglion cells and is incorporated into protein which is transported along the optic fibers at two different rates (*5, 7, 8*). Some of the transported protein arrives at the endings of the fibers within 24 hours after the injection, whereas the remainder moves more slowly, at the rate of about a millimeter per day. In the experiments here reported, the variations in the amount of radioactive protein in the lateral geniculate body, where many of the optic axons end, were closely followed by variations in the amount of radioactive material in the region of cortex to which the geniculate is connected. I believe that this provides evidence of the transfer of some substances from the optic axons to the geniculate neurons.

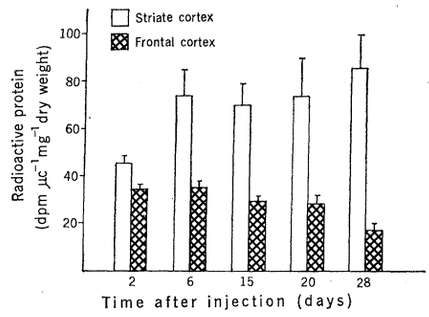

Fig. 1. Radioactive protein in striate and frontal cortex at various times after injection of [^{3}H]proline into the eye. Three animals were used for each time point; both eyes were injected and both hemispheres were sampled in each case. Vertical lines indicate the standard error of the mean. The difference between striate and frontal regions was statistically significant at a level of confidence exceeding 95 percent at every time point.

Thirty-three mice 2 to 6 months old and weighing 20 to 25 g were used. They were bred in the laboratory from C57BL/6j stock obtained from Jackson Laboratories. Under pentobarbital anesthesia, L-[2,3-^{3}H]proline (2 μc; specific activity, 45.7 c/mmole) in 2 μl of saline was injected into the posterior chamber of one or both eyes. At appropriate intervals after the injection the animals were decapitated under ether anesthesia, and the tissue content of radioactivity in samples of brain was determined by liquid scintillation counting after acid fixation of the tissue (*9*). In line with current biochemical practice, it has been tentatively assumed in the present study that the acid-insoluble material is mostly protein. For the samples of cortex, the results were expressed in disintegrations per minute (dpm) per microcurie injected per milligram dry weight of the sample. For the samples of lateral geniculate body the results were expressed in disintegrations per minute per microcurie for each whole geniculate.

Since the goal of the study was to identify material appearing in the visual cortex, it was important to recognize this area from external landmarks during the dissection. Seen from above, the striate cortex in the mouse occupies approximately the middle third of the hemisphere at its posterior pole and extends forward for about a third of the distance to the anterior pole (*10*). It was most convenient to use the medial and lateral borders of the inferior colliculus at the points at which they meet the posterior edge of the hemisphere to delimit the width of the sample taken from the striate region, about 1.5 mm in most cases; the length was taken to be about twice the width.

Beginning 8 hours after the injection of tritiated proline into the eye, this sample of cortex showed a consistently higher amount of radioactive trichloroacetic acid (TCA)–precipitable material, presumably protein, than an equivalent sample taken from the frontal pole of the hemisphere (Fig. 1). Thus, labeled protein accumulated in the striate region in excess of what would have been expected from local background incorporation in the cortex of labeled amino acid which had been absorbed from the eye by the bloodstream and then distributed throughout the brain.

If only one eye was injected with the isotope, the accumulation of labeled protein was confined to the contralateral visual cortex (*11*). This was not due to any disturbance produced by the act of injection, since the same result was obtained regardless of whether the unlabeled eye remained undisturbed or received an equivalent injection of unlabeled proline in saline. Thus, when the right eye was labeled and samples were taken from various cortical regions (Table 1), it was clear that the largest difference between the two sides of the brain was in the "striate" sample, defined by the landmarks indicated above. In the region medially adjacent, there was a smaller difference, which was also statistically significant. This region would contain the margin of the striate area and the parastriate area to which direct geniculate connections also exist (*12*). A still smaller difference ap-

Table 1. Radioactivity on the two sides of the brain 15 days after injection of [^{3}H]proline into the right eye (means ± standard error of the mean from six animals).

Structure	Radioactivity (dpm $\mu c^{-1}mg^{-1}$)		P (L − R = 0)
	Left side	Right side	
	Protein		
Striate cortex	85 ± 7	34 ± 2	< .001
Medial cortex	41 ± 2	24 ± 3	< .001
Lateral cortex	36 ± 4	29 ± 3	N.D.*
Anterior cortex	21 ± 3	24 ± 4	N.D.
Frontal cortex	25 ± 2	28 ± 2	N.D.
Lateral geniculate body †	2710 ± 540	260 ± 40	< .001
	TCA-soluble material		
Striate cortex	33 ± 13	15 ± 5	N.D.
Other cortex ‡		18 ± 2	< .05§
Lateral geniculate body †	15 ± 5	11 ± 3	N.D.

* No significant difference. † Radioactivity measured in disintegrations per minute per microcurie. ‡ All cortical samples from both sides, other than left striate ($n = 52$). § For comparison of left striate cortex with all other cortex.

peared in the region laterally adjacent to that defined as striate, but this was not statistically significant. The region immediately anterior to the striate sample and the region taken still more anteriorly from the frontal pole of the hemisphere did not show any difference between the two sides.

In addition to protein, the left striate cortex also showed a small accumulation of TCA-soluble material, presumably including free amino acid, polypeptides, and other small molecules (Table 1). In the lateral geniculate body, on the other hand, there was no significant difference in the TCA-soluble material on the two sides, although the difference in protein was very much higher than in the cortex.

Since the average dry weight of the cortical samples was 0.65 mg, we can calculate from the values of Table 1 that the difference between the left and right sides of the cortex consisted of 33 dpm/μc as protein in the striate sample (*13*), 10 dpm/μc as TCA-soluble material in the striate sample, and 11 dpm/μc as protein in the medial sample. Thus the total difference, of which about 80 percent was in protein, amounted to 2.2 percent of the accumulation (difference between left and right sides) in the lateral geniculate body.

The proportion of geniculate radioactivity appearing in the cortex remained constant throughout the period from 2 to 15 days after the injection (*14*), even though the geniculate radioactivity increased about threefold during this period due to the arrival of the slow component of transport. This suggests that the cortical material represented a constant proportion of both the fast and slow components arriving in the geniculate. One day after the injection, however, this proportion was only about half that seen at 2 days, indicating a lag in its arrival in the cortex. For the most rapidly appearing material the lag between geniculate and cortex was about 5 hours, which would be compatible with transport from geniculate to cortex at a rate approaching that of the fast transport in the primary optic fibers.

These observations are difficult to explain unless it is assumed that transneuronal transfer was occurring in the lateral geniculate body, with the labeled material passing from the optic axons into the geniculate neurons and then being transported along their axons to the visual cortex. The following alternative explanations can be considered.

1) A direct connection of the optic fibers to the cortex. There is no anatomical evidence for this. If such a pathway were postulated, it would be difficult to explain why TCA-soluble material would appear at optic terminals in the cortex but not in the geniculate.

2) An extracellular route from geniculate to cortex, either via the cerebrospinal fluid (*15*) or along the endoneurial spaces (*16*). This would not explain why the radioactivity arriving in the cortex is so strictly confined to the visual area.

3) Retrograde transport along the axons of cortical cells that end in the geniculate (*17*). Although there is at present little evidence for retrograde transport in the absence of damage to the axon (*18*), this remains a possibility that cannot be immediately dismissed, but not a highly probable one.

In the search for the trophic substance it has generally been assumed that what would be conveyed to the postsynaptic cell would probably be some kind of informational molecule, possibly of macromolecular size (*19*). However, an alternative idea that may not have received enough attention is that the materials responsible for the trophic effect might not be special molecules at all, but might be the metabolic breakdown products of the presynaptic cell, having a special effect because they were being delivered so close to the surface of the postsynaptic cell. Since the material that has been detected in the cortex consists largely of what appears to be protein, it would be essential to determine whether it had been transferred in its macromolecular form, or whether protein synthesis in the postsynaptic cell had occurred. The preparation here described makes these and other important investigations possible.

BERNICE GRAFSTEIN

Department of Physiology,
Cornell University Medical College,
New York, New York 10021

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4 January 1971; revised 25 February 1971